We compared uptake kinetics and aggregation kinetics in buffer and in cell culture medium at different Abeta 42 concentrations to test whether aggregation precedes uptake or vice versa. To compare the uptake of different Abeta species, pre-aggregated Abeta oligomers or small fibrils were added to the cells. We found that pre-aggregation accelerated the formation of intracellular aggregates, which suggests that Abeta oligomers and / or small fibrils may be taken up more rapidly than Abeta monomer.
In the future the form and location of intracellular Abeta will be monitored by high resolution fluorescence nanoscopy combined with atomic force microscopy.

2247-Pos Board B17
Biophysical Studies on Protein Aggregation and Amyloid Fibril Formation
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Protein misfolding leading to aggregation and amyloid fibril formation has been implicated in a number of debilitating human disorders. The primary causative agent is commonly identified to be an aberrant misfolded-form of a protein that self-assembles into oligomers which eventually lead to the formation of ordered cross-β-rich amyloid fibrils. The transiently-populated oligomeric intermediates enroute to amyloid assembly have drawn considerable attention owing to their higher cytotoxicity compared to that of mature amyloid fibrils. Our efforts are directed towards unraveling the mechanisms of amyloid fibrillation using a diverse array of biophysical tools involving steady-state and time-resolved fluorescence, circular dichroism, Raman spectroscopy, dynamic light scattering, electron microscopy and atomic force microscopy [1-3]. Our recent findings on aggregation of an all α-helical protein namely serum albumin revealed that low pH-induced partially-unfolded, molten-globule-like conformers associate to form obligatory oligomeric intermediates that serve as precursors to amyloid fibrils. Comparison of the kinetics of protein conformational- and size changes using multiple structural probes in-tandem indicated that oligomerization followed by conformational conversion leads to the formation of β-rich fibrils. Recently, we have extended our biophysical studies to other proteins such as ovalbumin. I will also discuss our recent results on chain collapse and oligomerization of intrinsically disordered proteins that are capable of forming amyloid fibrils.
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**Equal contribution.*

2248-Pos Board B18
Single Molecule Fluorescence Studies of Amyloid-Beta 1-42 Aggregation
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The proteolytic cleavage of the transmembrane amyloid precursor protein (APP) produces amyloid-β peptides (Aβ) that vary from 38 to 43 amino acids in length. Two of these peptides, $A\beta_{1\text{-}40}$ and $A\beta_{1\text{-}42}$, are the major components of the extracellular amyloid plaques characteristic of Alzheimer's disease (AD). Within these plaques, the Aβ is found aggregated into long polymeric assemblies rich in β-sheet structure that are known as amyloid fibrils. Although the correlation between plaque load and disease severity is poor there is strong evidence that small soluble oligomers of Aβ formed during the early stages of the aggregation process are the agents of AD-associated neurotoxicity (1). Single molecule fluorescence techniques have the potential to resolve the size and structural heterogeneity of these oligomers, which are often difficult to discern by ensemble methods. Most importantly, they allow the characterisation of small oligomeric species at the nucleation stage of the aggregation as the structures of amyloid seeds remain ambiguous (2). Equimolar mixtures of $A\beta_{1\text{-}42}$ singly labelled with either HiLyteFluor-488 or HiLyteFluor-647 were studied using single molecule fluorescence confocal microscopy and FRET, allowing the characterisation of oligomers present during aggregation of monomers and disaggregation of fibrils. Additionally, we have extended our single-molecule studies to examine the species formed during the co-aggregation of $A\beta_{1\text{-}40}$ and $A\beta_{1\text{-}42}$ to understand the interaction at physiological concentrations and ratios. The thorough detection and characterisation of these potentially toxic oligomeric species provides a basis with which to screen therapeutic agents and other modulators of aggregation *in vitro* which could inform *in vivo* studies in the future.
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2249-Pos Board B19
Replica Exchange Statistical Temperature Molecular Dynamics Simulations of Peptide Dimerization
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We present a new computer simulation algorithm called Replica Exchange Statistical Temperature Molecular Dynamics that combines the multicanonical sampling technique of Statistical Temperature Molecular Dynamics with temperature replica exchange. This algorithm is related to Wang-Landau sampling but uses a dynamical update of the density of states to achieve flat energy sampling within a replica-dependent temperature range. This algorithm is used to investigate the thermodynamics of dimerization of two polypeptide chains. Three two-peptide systems are investigated: two hydrophobic peptides, two hydrophilic peptides, and one of each. Each monomer is modeled using a coarse-grained peptide model that has an α-helix as the lowest energy configuration. For each dimer system, interesting folding behavior is observed. It is found that at low temperatures, both peptides are helical and the lowest-energy configuration maximizes inter-peptide contact; at high temperatures, both peptides are random coils; and at intermediate temperatures, one peptide is folded and the other unfolded. Formation of the peptide dimer causes one peptide to fold at a *higher* temperature than an isolated monomer and the other to fold at a *lower* temperature than an isolated monomer. Dimerization causes one peptide to become more stable and the other peptide to become less stable. It is also shown that at intermediate temperatures, neither peptide adopts a random coil configuration: the helical peptide induces a conformational change in the unfolded peptide. The Potential Energy Surface (PES) is also determined for each dimer and the effect of hydrophobic/hydrophilic nature of the peptide on the PES is discussed.

2250-Pos Board B20
Protein Structure in Amorphous Solid Phase
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Protein-based medicines often require freezing or stabilization in carbohydrate glasses for storage prior to use. The structural stability of these proteins is of the great importance in the conditions required for pharmaceutical purposes. Problems involving aggregation and stability of the protein in freeze-dried formulations are of the challenges for the pharmaceutical industry.
Small-angle neutron scattering (SANS) is uniquely qualified to study the structure of proteins in the liquid and solid phases that are biotechnologically relevant for proteins. The structural and conformational changes of a model protein, lysozyme, during the destabilizations in water- ice and carbohydrates systems were studied using SANS and MD simulations. X-ray diffraction measurements were performed to verify existence of cubic and hexagonal ice structures in protein-ice system. Measurements and modelling efforts to understand protein structural changes will be discussed and the interaction distances measured by SANS and proposed model protein structures in different carbohydrate glasses will be compared.

2251-Pos Board B21
Osmolyte Effect on Aggregation of ß-Lactoglobulin Amyloid-Prone Peptides by Explicit Molecular Dynamics Simulation
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Whereas the toxicity of pathogenic amyloids relies on protein misfolding, other nonpathogenic or even functional amyloid structures can regulate physiological activity in a number of domains. Understanding the structural transition within this class of amyloids will provide insights into the general mechanism for ordered to agregation-dependent transitions. We have performed explicit molecular dynamics simulations using GROMACS with GROMOS53A6 force field and SPC water model. We have investigated 6 and 12 peptides of sequence: Ac-y^{146}HIRLSFN^{152}NH_2, from bovine ß-lactoglobulin; theses peptides are known to display high aggregation propensity under specific experimental conditions (5 M urea). We have shown by MD that the peptides form in water and in 5 M urea, in less than 100 ns, a structural aggregate displaying antiparallel ß-sheets, with an hydrophobic core protected from water. Furthermore, we have examined the effect of two different osmolytes (2.5 and 5 M Urea and 1.5 M Trehalose) on the nature of the interactions favoring the ß-structure of

the peptidic-aggregates. The addition of each osmolyte or a mixture of both increases the conformational stability of the six peptide amyloid-prone aggregate. All the studied osmolytes, decrease the number of peptide-water hydrogen bonds, increase the peptide-osmolyte hydrogen bonds, while the number of the intrapeptide hydrogen bonds remains almost unchanged. This result is in good agreement with the suggested mechanism of osmolytes replacing water molecules on the peptide aggregate surface. In contrast our molecular dynamics simulation has uncovered that the conformational stability of the 12 peptide aggregate was very similar in water, in presence of each osmolyte or a mixture of both. This effect is most probably due to the higher content of intrapeptide hydrogen bonds existing in the hydrophobic core formed within the 12 peptide aggregate.

Protein Conformation II

2252-Pos Board B22

Conformational Dynamics of the *E. Coli* SOS Mutagenesis Protein UmuD

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The SOS response in *Escherichia coli* involves the induction of at least 57 genes in response to DNA damage, including the *umuD* gene. $UmuD_2$ is a homodimer of 139-amino acid subunits that interacts with RecA/ssDNA nucleoprotein filaments, resulting in cleavage of its N-terminal 24-amino acids to yield $UmuD'_2$. The full-length form UmuD plays a role in a primitive DNA damage checkpoint and prevents mutagenesis, whereas the cleaved form facilitates mutagenesis by Y family DNA polymerase UmuC. The goal of our research is to determine the conformation and dynamics of UmuD in order to understand its regulatory role in response to DNA damage. $UmuD_2$ and $UmuD'_2$ are both exceptionally tight dimers, with picoMolar dissociation constants. However, we find that a monomeric variant of UmuD maintains essentially all biological roles of $UmuD_2$. This surprising finding suggests that UmuD can cleave in either a *cis* or *trans* conformation. By forming alternate dimeric forms of UmuD, we determined that cleavage in *trans* is more efficient than cleavage in *cis*. $UmuD_2$ and $UmuD'_2$ readily exchange monomers to form the heterodimer UmuDD', which is the most stable dimeric form. We are investigating the kinetics and mechanism of exchange of UmuD dimers and the UmuDD' heterodimer by using FRET assays and native PAGE. The different dimeric forms of the *umuD* gene products exchange on the minute time scale, and each dimer can continue to exchange once formed. Designed site-directed mutations are expected to allow us to determine the mechanism of UmuD dimer exchange.

2253-Pos Board B23

Persistent α-Helical Content and Local Helical Structural Fluctuations from a Molten Globule to Ordered Peptide Transition

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Structural information about proteins can provide valuable insight into folding mechanisms and dynamics, giving us information about protein function and interactions within biological systems. Upon interaction with various moieties, proteins have the potential to undergo a variety of conformational changes. Extensive studies have been done using deep UV resonance Raman (dUVRR) spectroscopy to study the secondary structure of proteins. Here, we present the results of our experiments, where we simultaneously monitored both secondary and tertiary structure of de novo synthesized protein, HP7. HP7 is a four-helix bundle that retains its secondary structure while altering its tertiary structure dependent upon the attachment of a heme group. In studying the apo- and holo-proteins, we determine that dUVRR spectroscopy is a promising tool for the simultaneous study of both protein secondary and tertiary structure.

2254-Pos Board B24

Probing the Temperature Dependence of Structure and Dynamics of Thermophilic Lactate Dehydrogenase from Th. Maritima

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Thermophilic lactate dehydrogenase (LDH) from Thermotoga maritima demonstrates a drastic increase in Km with increased temperature, compared with that of mesophilic LDH. To investigate such difference and the temperature adaptation of proteins, FTIR is used to probe the structure dependence on the temperature by monitoring amide-I band and CO stretch of oxamate in the ternary complex. It has been found that, although protein denaturing are not observed with an increased temperature up to 80 C (the temperature for optimum growth of Th. maritima), the protein structure as reported by T. Dams et al., shows difference between room temperature and 80 C. FRET and laser induced temperature jump techniques are employed to study the dynamics of ligand-protein complexes of TmLDH on the nanosecond time scale.

2255-Pos Board B25

Heterospectral 2D Correlation Analysis of the Redox Induced Two-Step Conformational Transition of Cytochrome C

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Redox proteins are the main components of the respiratory chain. Even though their structure is known in great detail, information about structure-function relationships is still sparse. Vibrational spectroscopy is well designed to fill this gap Raman- and Infrared-Spectroscopy yielding complementary information, they are usually applied separately. Information about heme centres and peptide structures are thus obtained, which are Raman and infrared-active, respectively. If, for example the protein is immobilized on an electrode, changes of the protein-backbone and the heme-centre may be observed as a function of an applied potential. To fully understand the mechanism of the interaction of heme-centre with the protein-backbone information obtained by both techniques have to be correlated.

Cytochrome c (cc) containing a Raman-active redox-centre, surrounded by the infrared-active backbone, has been used as a benchmark system to introduce the concept of hetero 2D correlation spectroscopy.

The redox transition of the heme group as a function of potential could be correlated with conformational changes of the following peptide groups 32-40, whereas other groups such a 14-19 and 57-59 do not change simultaneously. Hence we can conclude that changes in the protein backbone as a function of the redox transition occur in a two-step process. This is consistent with MD calculations showing that the a-helical structure is elongated during oxidation whereas a β-structure is formed.

2256-Pos Board B26

Investigations of the ATPase Mechanism of HSP70 Molecular Chaperones upon Substrate Binding using Protein Engineering and Computational Techniques

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Hsp70 chaperones play mportant roles in cells including protein folding, trafficking, degradation and enabling survival under stress conditions. DnaK is an *Echerichia coli* Hsp70 homolog comprising a 44 kDa ATPase domain (NBD) and a 25 kDa substrate-binding domain (SBD). DnaK has two nucleotide substrate-affinity states: In the ATP-bound low substrate-affinity state, substrate binding and release occur rapidly, whereas in the ADP-bound high substrate-affinity state, slower substrate binding and release kinetics are observed. Communication between the two domains is essential for chaperone function and is mediated via a conserved hydrophobic linker region (^{384}GDVKDVLLL^{392}). Previous studies showed that when this flexible linker interacts with the ATPase domain, which was studied by the construct containing the entire linker, DnaK(1-392), an enhanced ATPase rate is observed compared to the construct lacking the conserved ^{389}VLLL^{392} linker region, DnaK(1-388). This observation suggests that structural rearrangements caused by linker docking adopt the ATPase domain in a closed conformation, leading to an enhanced, pH-dependent ATPase activity. Here, our aim is to delineate the residues that are responsible for the linker induced conformational rearrangements. In that line, using molecular dynamic simulations we identified two sets of amino acids at the lobe interface of the ATPase domain that might be critical in the stabilization of the domain in the so called ''open'' and ''closed'' conformations. We made point mutations for these sites on both DnaK(1-392) and DnaK(1-388) constructs, and studied the structural and functional effects of these residues on the ATPase domain using pH varied stability measurements by circular dichroism and activity measurements as a function of pH, respectively. Our experimental results also point to the significance of these residues in the domain rearrangements when triggered by linker binding.

2257-Pos Board B27

Dynamic Motions of the G Protein Subunit G Alpha I1 While Complexed to the GEF Ric-8A

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Canonical G-protein signaling pathways are activated when agonist-bound G protein coupled receptors (GPCR), acting as guanine nucleotide exchange factors (GEF), promote the exchange of GDP for GTP on Gα subunits present in GαGDP:Gβγ heterotrimers. GEFs catalyze product release and substrate